REVIEWED
By Celena Shrum at 11:41 am, Jul 13, 2022

TS 7/5/2022

Worklist: 6021

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION	
M2022-1436	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2022-2016	2	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2022-2116	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2022-1195	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2022-1725	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2022-1760	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2022-1762	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2022-1764	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2022-1777	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2022-1795	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2022-1797	1	BCK	AM 27 Blood THC Quant by LC-QQQ	

TS

AM# 27: Quantitation of THC and Metabolites in Blood and Urine by LC-MS/MS

Extraction Date: 07/06/2022
Plate lot#: 220309
Mobile phase A: 0.1% Formic Acid in LCMS Water
Blank Blood Lot: Lampire 20L20723
Column: UCT Selectra DA 100 x 2.1mm 3um

Analyst: Tamara Salazar
Plate Retest Date: 09/09/2022
Mobile phase B: 0.1% Formic acid in Acetonitrile
Blank Urine Lot: N/A
LCMS-QQQ ID: 069901

Pre-Analytic:

☒ 1. Check levels of mobile phases and needle wash refill as needed. Ensure waste is not full.
☒ 2. Ensure correct column is installed and begin mobile phase flow allow to equilibrate ~ 30 minutes.

Analytic:

☒ 1. Remove standards, plate, controls, and samples from cold storage. Allow to reach room temperature.
☐ 2. ~~Urine hydrolysis: add 1.5mL urine to blank plate, add 250µl 1N KOH. Shake and incubate at 40 degrees for 15 minutes.~~
☒ 3. Using a calibrated pipette, add **1000µl blood and urine (if applicable) (calibrated pipette)** into the appropriate wells of analytical (standards) plate. **Pipette ID:** *42*
☒ 4. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
☒ 5. Add **500µL of 0.1% formic acid in water to blood samples, and 500µL of saturated phosphate buffer to urine samples** in the wells of the analytical plate.
☒ 6. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
☒ 7. Transfer **700-800µL of blood+acid or urine+acid** mixture to corresponding wells of SLE+ plate. Amount transferred: *750µL*
☒ 8. Apply positive pressure for approx. 10-15 seconds (or until no liquid remains on top of sorbent). ***(Load at 85-100 PSI- Selector to the right)***
☒ 9. Wait 5 minutes.
☒ 10. Add **2.25mL MTBE. (*Add in 3 increments of 750uL)***
☒ 11. Wait 5 minutes.
☒ 12. Apply positive pressure for approx. 15 seconds. ***(10-15 PSI- Selector to the left).***
☒ 13. Add **2.25mL Hexane. (*Add in 3 increments of 750uL)***
☒ 14. Wait 5 minutes.
☒ 15. Apply positive pressure for approx. 15 seconds. ***(10-15 PSI- Selector to the left).***
☒ 16. Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approx. 35°C.
☒ 17. Reconstitute in **100µL 100% MeOH** and heat seal plate with foil. Place in autosampler and run worklist.

Post-Analytic

☒ 1. Create batch and process data.
☒ 2. Make any necessary integration changes, Curve weighting of Linear 1/x with r^2 values ≥0.98 for each analyte
☒ 3. RT +/- 3% or 0.100 min, whichever is greater, +/- 20% Accuracy for greater than (+/- 30% for 10ng/ml or less). Ion ratios must be within +/- 20% of the averaged calibrators
☒ 4. Case samples with calculated concentrations for THC at 1ng/mL or greater and OH-THC at 3ng/mL or greater may be reported quantitatively (blood only). Calculated concentrations for carboxy-THC of 5ng/mL may be reported qualitatively. Samples with a THC or OH-THC response over 50 ng/mL will be reported out as greater than 50 ng/mL.
☒ 5. Did all QCs pass for each analyte? (if not, describe in comments section)
☒ 6. Enter QCs into control charting.
☒ 7. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS:
THC-OH not evaluated due to possibly interfering peak.
THC-COOH 5-100 - calibrator 7 dropped due to accuracy

TS

	1	2	3	4	5	6
A	IS + Cal. 1	IS + QC_1	IS + Sample	IS + Sample	P2022-1762-1	IS + QC_1
B	IS + Cal. 2	IS + Sample	IS + Sample	IS + Sample	P2022-1760-1	IS + Cal. 7
C	IS + Cal. 3	IS + Sample	IS + Sample	IS + Sample	P2022-1725-1	IS + Cal. 6
D	IS + Cal. 4	IS + Sample	IS + Sample	P2022-1195-1	M2022-2116-1	IS + Cal. 5
E	IS + Cal. 5	IS + Sample	IS + Sample	P2022-1797-1	M2022-2016-2	IS + Cal. 4
F	IS + Cal. 6	IS + Sample	IS + Sample	P2022-1795-1	M2022-1436-1	IS + Cal. 3
G	IS + Cal. 7	IS + Sample	IS + Sample	P2022-1777-1	Neg Blood	IS + Cal. 2
H	IS + QC_1	IS + Sample	IS + Sample	P2022-1764-1	IS + QC_1	IS + Cal. 1

All wells to contain 100 µl of residual DMSO

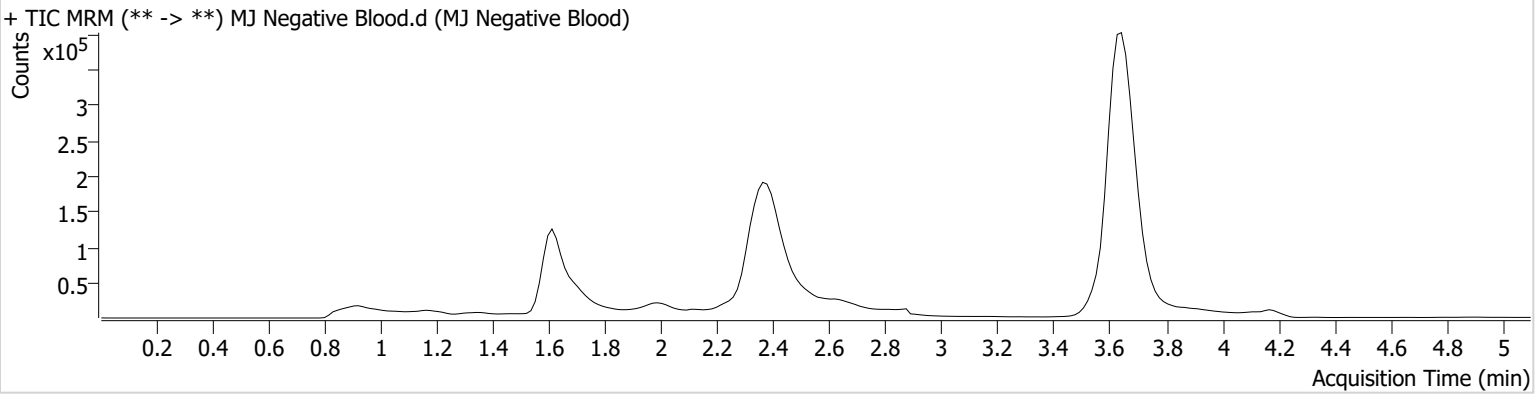
TS

AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\070622 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/12/2022 7:51:05 AM

Instrument	Falco (069901)	**Data File**	MJ Negative Blood.d
Type	Sample	**Sample**	MJ Negative Blood
Acq. Method	AM 27 THCQ.m	**Operator**	Tamara Salazar
Sample Position	P1-G5	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/7/2022 5:06:28 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.693	112449	∞	2.1 **Low**	17.57	584181	1.4941	ng/ml **Low**

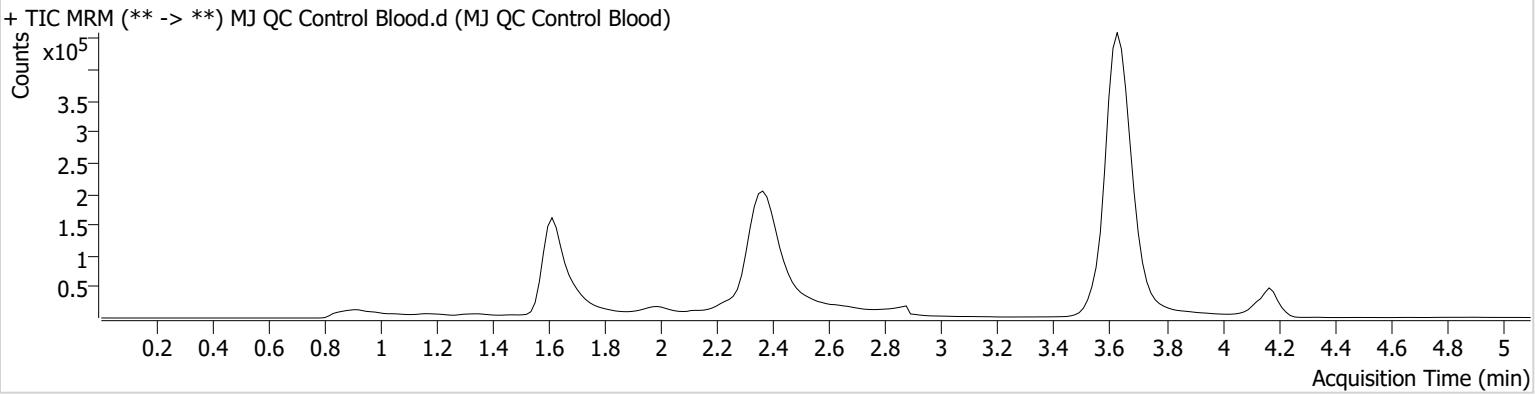
TS

AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\070622 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/12/2022 7:51:05 AM

Instrument	Falco (069901)	**Data File**	MJ QC Control Blood.d
Type	QC	**Sample**	MJ QC Control Blood
Acq. Method	AM 27 THCQ.m	**Operator**	Tamara Salazar
Sample Position	P1-A6	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/7/2022 4:51:16 AM		
Sample Info.			

Sample Chromatogram



	Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
	THC-COOH	1.655	37723	∞	66.5	450.55	136870	14.8456	ng/ml
*	~~THC-OH~~	~~1.693~~	~~122690~~	~~∞~~	~~5.8~~	~~35.37~~	~~524078~~	~~3.2555~~	~~ng/ml~~
	THC	3.646	120176	∞	29.2	∞	2926973	4.8031	ng/ml

*Compound not evaluated.

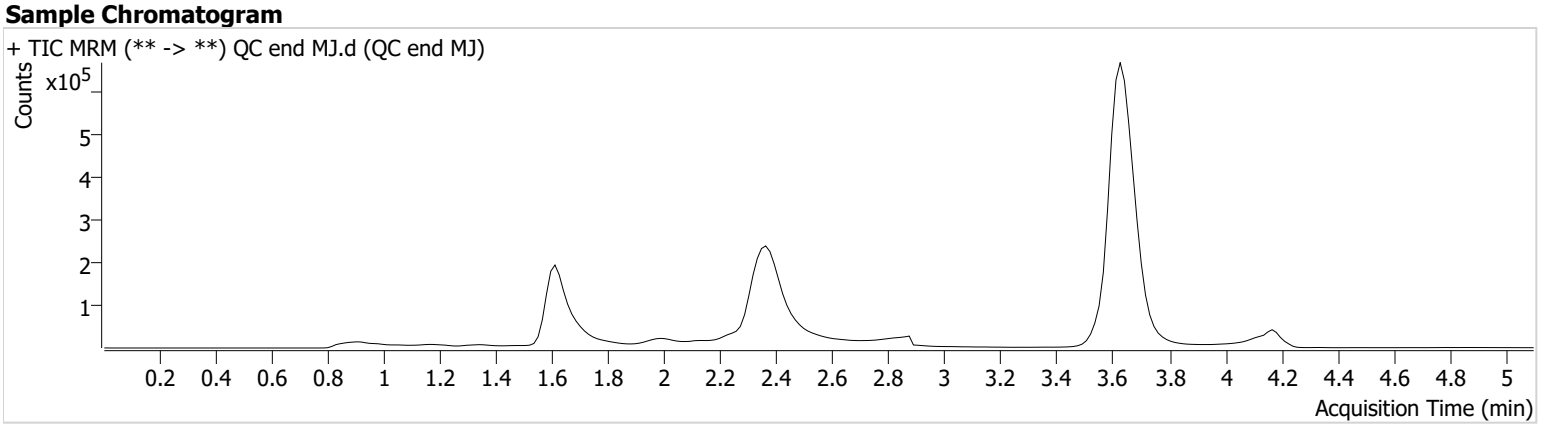
TS

AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\070622 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/12/2022 7:51:05 AM

Instrument	Falco (069901)	**Data File**	QC end MJ.d
Type	QC	**Sample**	QC end MJ
Acq. Method	AM 27 THCQ.m	**Operator**	Tamara Salazar
Sample Position	P1-A6	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/7/2022 8:09:11 AM		
Sample Info.			

Sample Chromatogram



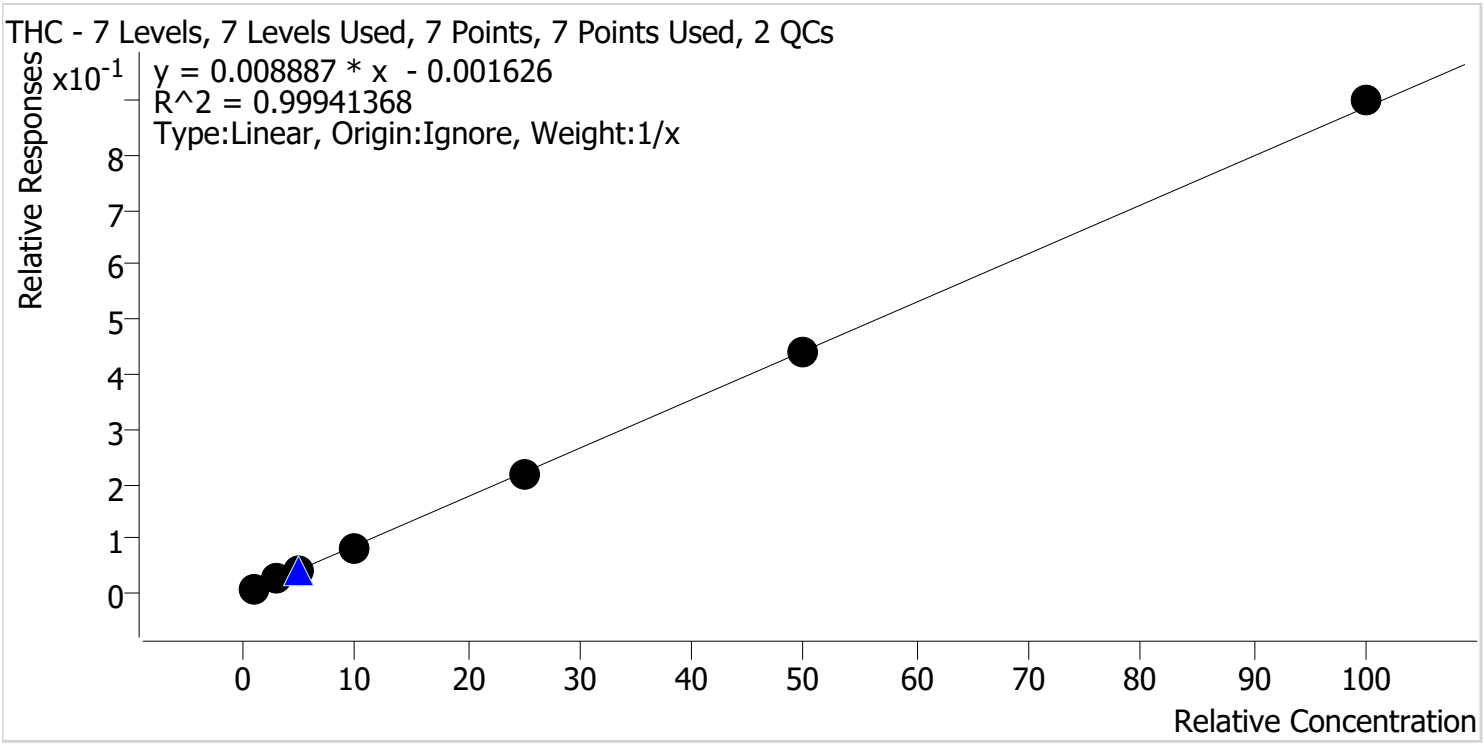
	Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
	THC-COOH	1.640	42916	∞	68.0	139.45	156352	14.7938	ng/ml
*	~~THC-OH~~	~~1.693~~	~~137436~~	~~∞~~	~~7.1 **High**~~	~~124.94~~	~~628691~~	~~2.5995~~	~~ng/ml **Low**~~
	THC	3.646	172261	∞	28.9	∞	4149677	4.8542	ng/ml

*Compound not evaluated.

TS

AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results	D:\MassHunter\Data\2022\AM 27-28\070622 AM 27 28 TS\QuantResults\AM 27.batch.bin
Last Cal. Update	7/12/2022 7:51 AM
Analyst Name	ISP\datastor
Analyte	THC
Internal Standard	THC-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✔	1.0	1.1	113.7
Cal 2 MJ	2	✔	3.0	2.9	97.4
Cal 3 MJ	3	✔	5.0	4.8	95.3
Cal 4 MJ	4	✔	10.0	9.5	95.2
Cal 5 MJ	5	✔	25.0	24.2	97.0
Cal 6 MJ	6	✔	50.0	50.0	100.1
Cal 7 MJ	7	✔	100.0	101.4	101.4

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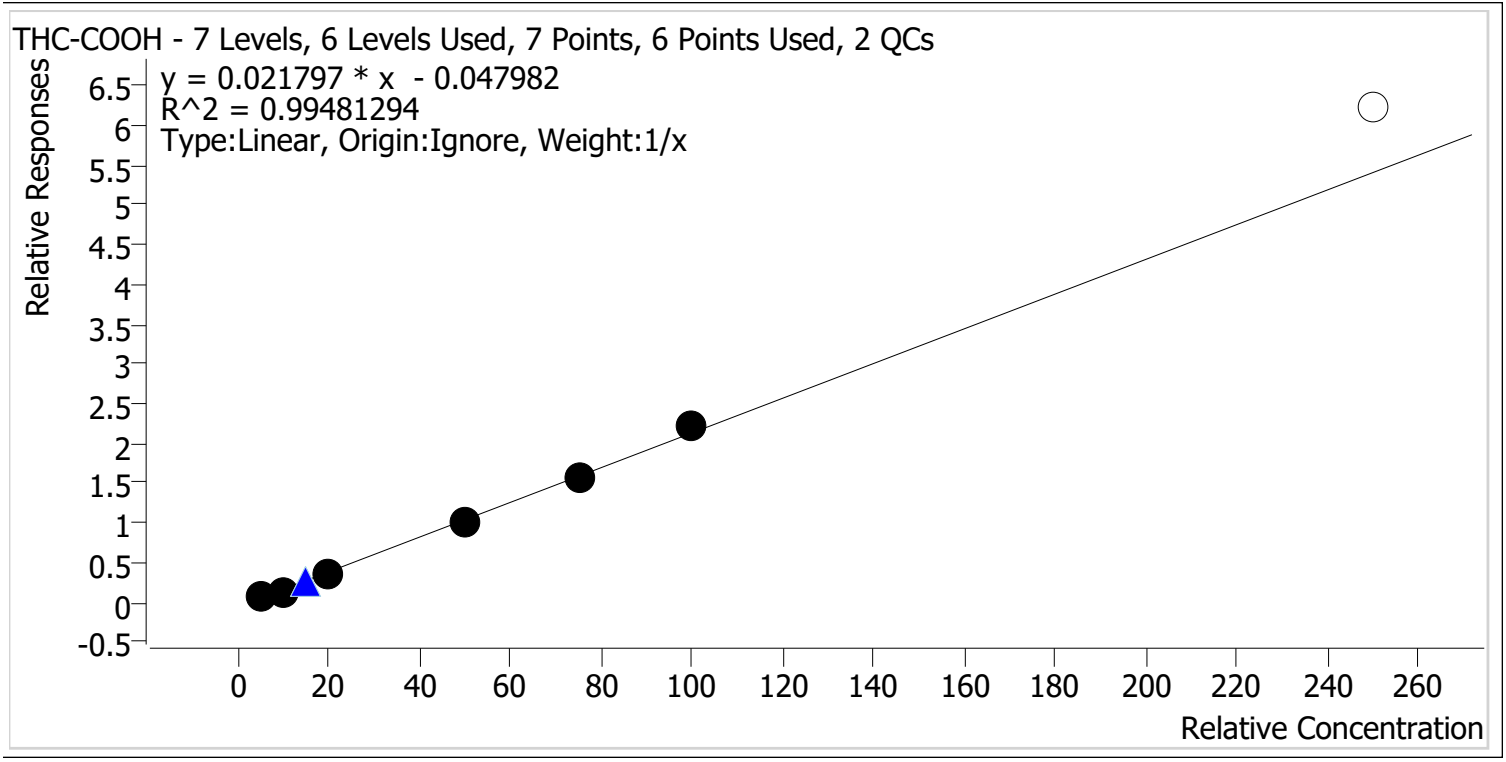
AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results	D:\MassHunter\Data\2022\AM 27-28\070622 AM 27 28 TS\QuantResults\AM 27.batch.bin
Last Cal. Update	7/12/2022 7:51 AM
Analyst Name	ISP\datastor
Analyte	THC-COOH
Internal Standard	THC-COOH-D9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✔	5.0	6.2	123.1
Cal 2 MJ	2	✔	10.0	8.6	86.4
Cal 3 MJ	3	✔	20.0	18.0	89.8
Cal 4 MJ	4	✔	50.0	48.7	97.4
Cal 5 MJ	5	✔	75.0	74.4	99.1
Cal 6 MJ	6	✔	100.0	104.2	104.2
Cal 7 MJ	7	✗	250.0	287.4	115.0

Calibrator 7 dropped due to accuracy.

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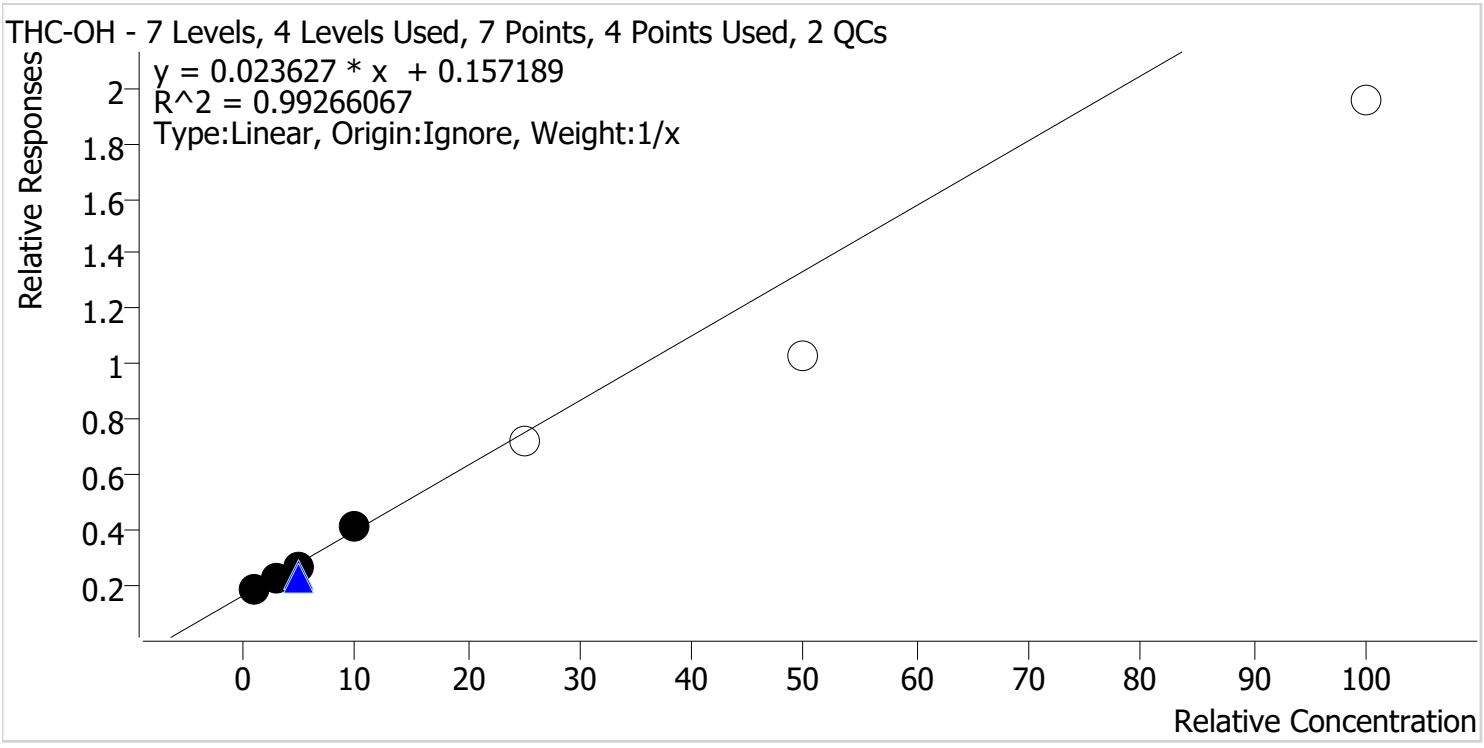
AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\070622 AM 27 28 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 7/12/2022 7:51 AM
Analyst Name ISP\datastor
Analyte THC-OH **Internal Standard** THC-OH-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✔	1.0	1.1	108.4
Cal 2 MJ	2	✔	3.0	2.8	95.0
Cal 3 MJ	3	✔	5.0	4.6	92.0
Cal 4 MJ	4	✔	10.0	10.5	104.7
Cal 5 MJ	5	✗	25.0	23.8	95.3
Cal 6 MJ	6	✗	50.0	36.6	73.2
Cal 7 MJ	7	✗	100.0	76.1	76.1

*Compound not evaluated.

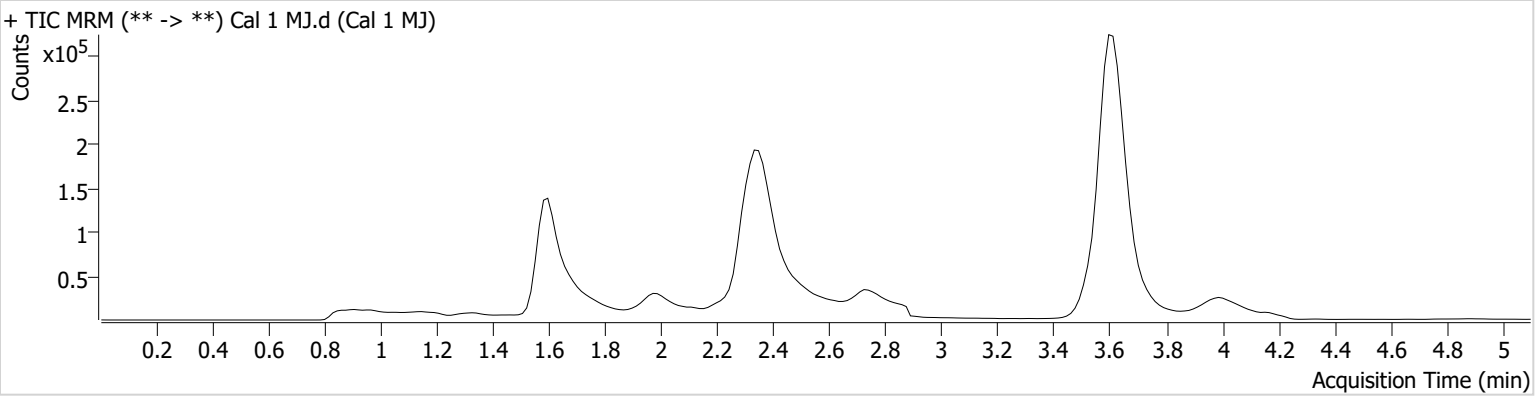
TS

AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\070622 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/12/2022 7:51:05 AM

Instrument	Falco (069901)	**Data File**	Cal 1 MJ.d
Type	Cal	**Sample**	Cal 1 MJ
Acq. Method	AM 27 THCQ.m	**Operator**	Tamara Salazar
Sample Position	P1-H6	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/7/2022 3:50:16 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-COOH	1.625	11326	∞	66.5	190.16	131373	6.1564	ng/ml
THC-OH	1.678	95464	222.99	3.6 **Low**	16.51	522263	1.0835	ng/ml **Low**
THC	3.616	19018	∞	35.4	∞	2242597	1.1372	ng/ml

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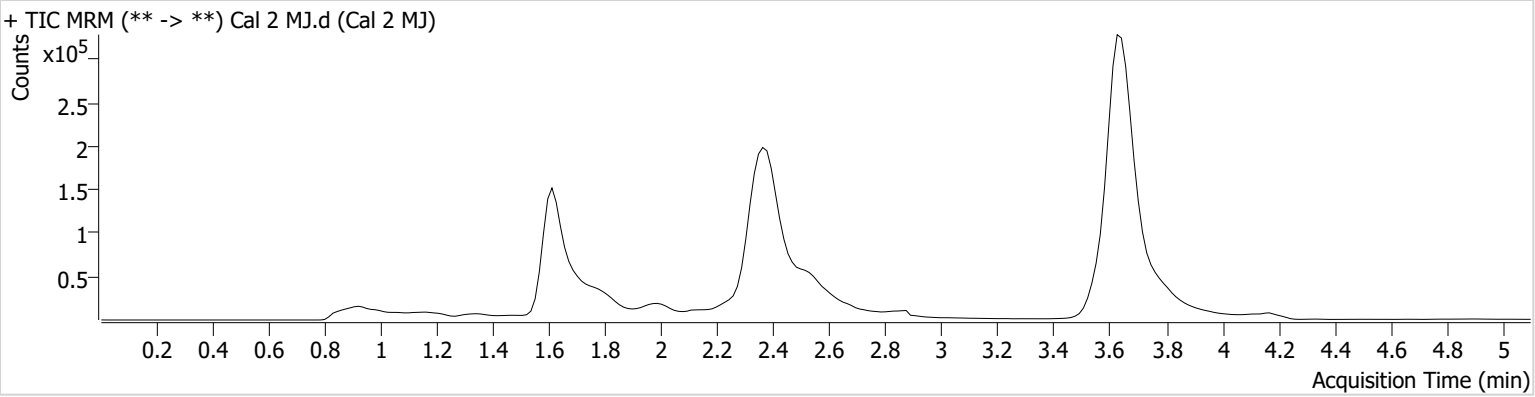
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\070622 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/12/2022 7:51:05 AM

Instrument	Falco (069901)	**Data File**	Cal 2 MJ.d
Type	Cal	**Sample**	Cal 2 MJ
Acq. Method	AM 27 THCQ.m	**Operator**	Tamara Salazar
Sample Position	P1-G6	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/7/2022 3:58:01 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.		
THC-COOH	1.655	18585	∞	79.6	∞	132474	8.6375	ng/ml	
THC-OH	1.693	126543	∞	4.5	10.93	563692	2.8485	ng/ml	Low
THC	3.646	58139	∞	30.3	∞	2388727	2.9217	ng/ml	

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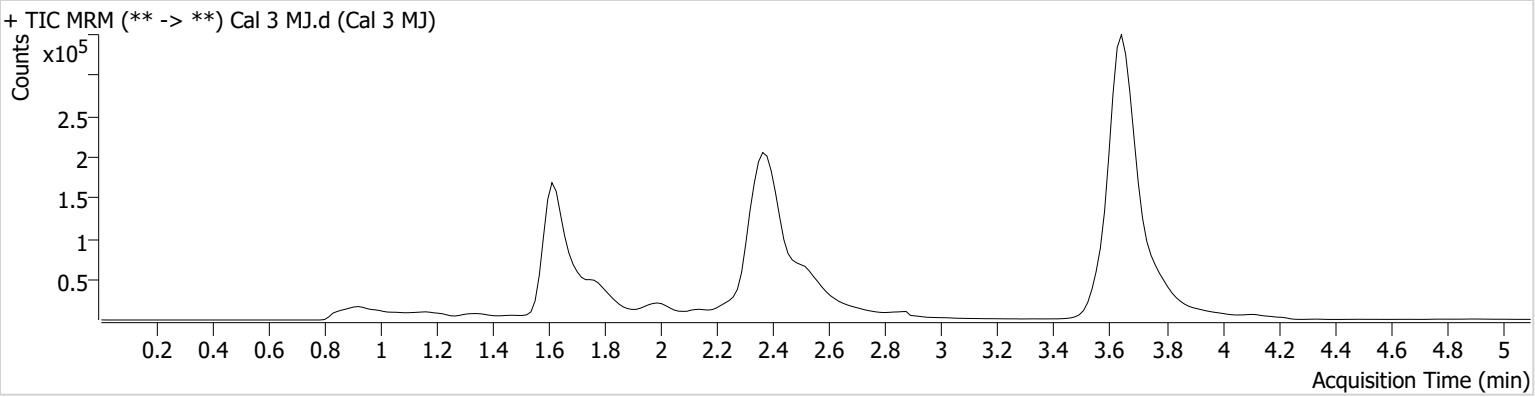
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\070622 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/12/2022 7:51:05 AM

Instrument	Falco (069901)	**Data File**	Cal 3 MJ.d
Type	Cal	**Sample**	Cal 3 MJ
Acq. Method	AM 27 THCQ.m	**Operator**	Tamara Salazar
Sample Position	P1-F6	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/7/2022 4:05:37 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-COOH	1.655	48238	∞	71.1	∞	140516	17.9503	ng/ml
THC-OH	1.693	154008	∞	5.1	25.13	579161	4.6019	ng/ml
THC	3.646	101616	∞	29.0	∞	2496766	4.7627	ng/ml

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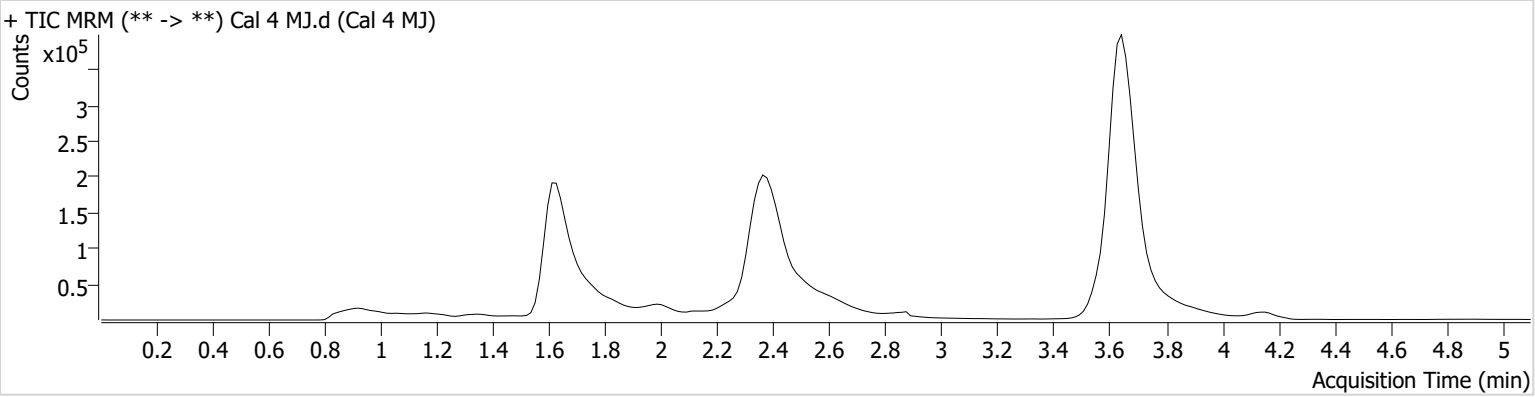
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\070622 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/12/2022 7:51:05 AM

Instrument	Falco (069901)	**Data File**	Cal 4 MJ.d
Type	Cal	**Sample**	Cal 4 MJ
Acq. Method	AM 27 THCQ.m	**Operator**	Tamara Salazar
Sample Position	P1-E6	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/7/2022 4:13:13 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-COOH	1.655	141496	∞	63.2	∞	139586	48.7060	ng/ml
THC-OH	1.693	206955	∞	8.4 **High**	104.63	511671	10.4661	ng/ml
THC	3.646	216746	∞	28.8	1346.46	2612197	9.5198	ng/ml

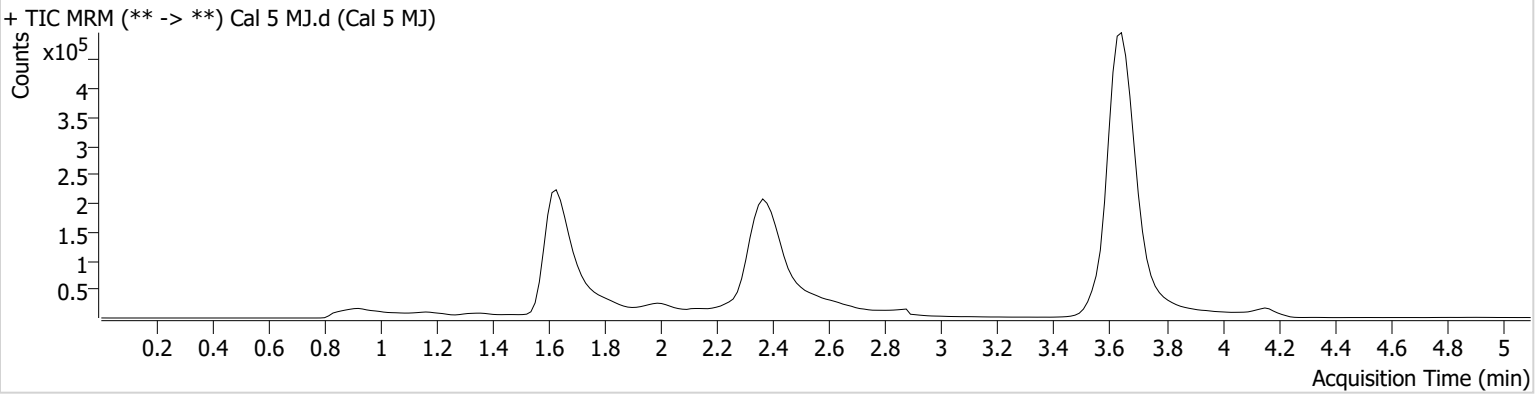
TS

AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\070622 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/12/2022 7:51:05 AM

Instrument	Falco (069901)	**Data File**	Cal 5 MJ.d
Type	Cal	**Sample**	Cal 5 MJ
Acq. Method	AM 27 THCQ.m	**Operator**	Tamara Salazar
Sample Position	P1-D6	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/7/2022 4:20:49 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-COOH	1.655	209915	∞	61.9	578.39	133475	74.3517	ng/ml
THC-OH	1.618 Low	348144	∞	10.5 High	442.86	483279	23.8370	ng/ml
THC	3.646	602311	∞	28.0	∞	2816871	24.2438	ng/ml

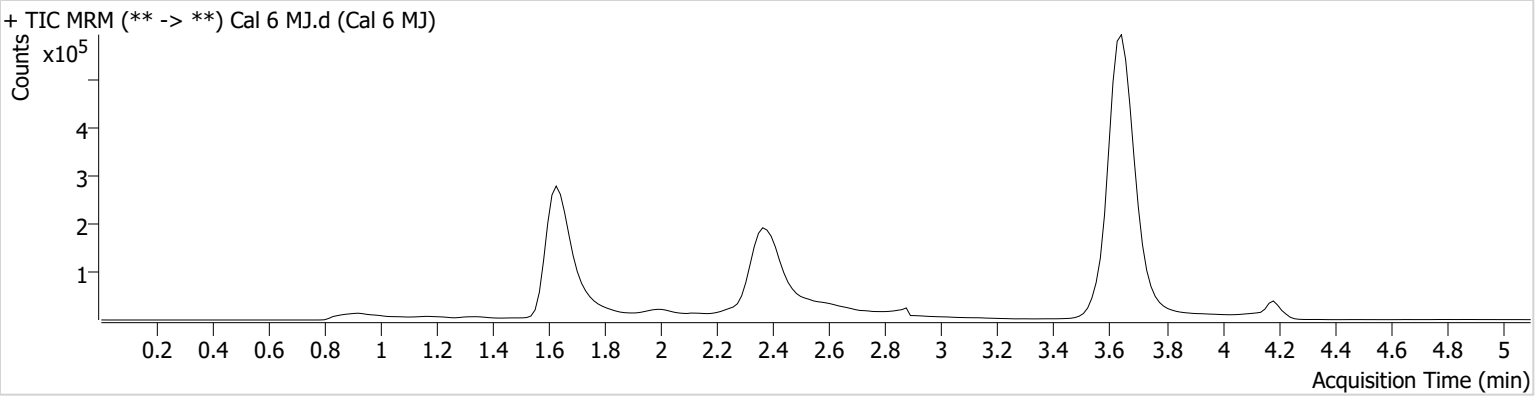
TS

AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\070622 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/12/2022 7:51:05 AM

Instrument	Falco (069901)	**Data File**	Cal 6 MJ.d
Type	Cal	**Sample**	Cal 6 MJ
Acq. Method	AM 27 THCQ.m	**Operator**	Tamara Salazar
Sample Position	P1-C6	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/7/2022 4:28:25 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-COOH	1.655	282185	∞	60.1	∞	126923	104.1980	ng/ml
THC-OH	1.618 Low	517168	∞	11.6 High	∞	505900	36.6146	ng/ml
THC	3.646	1136204	∞	27.6	∞	2564273	50.0425	ng/ml

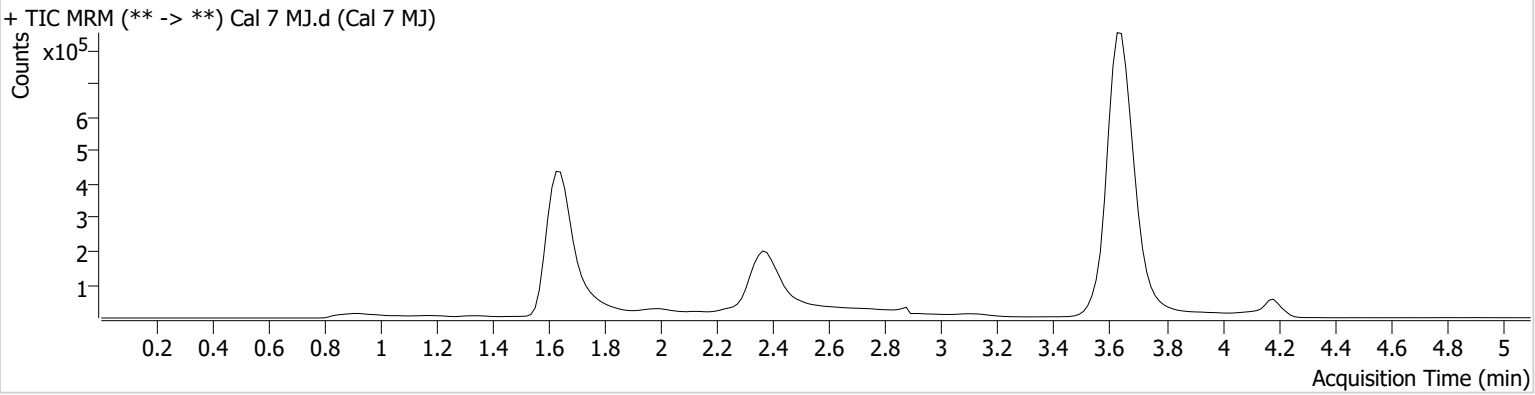
TS

AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\070622 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/12/2022 7:51:05 AM

Instrument	Falco (069901)	**Data File**	Cal 7 MJ.d
Type	Cal	**Sample**	Cal 7 MJ
Acq. Method	AM 27 THCQ.m	**Operator**	Tamara Salazar
Sample Position	P1-B6	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/7/2022 4:36:01 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-COOH	1.655	682740	∞	59.9	∞	109834	287.3774	ng/ml
THC-OH	1.618 Low	910929	∞	12.6 High	6018.05	465752	76.1272	ng/ml
THC	3.646	2438880	∞	27.6	∞	2712139	101.3722	ng/ml